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EFFECT OF PINEAL EXTRACTS AND HYPOTHALAMIC LESIONS ON THE GONADS OF THE GOLDEN HAMSTER

A DISSERTATION

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ABSTRACT

The hamsters in this investigation were placed in three groups. Group I contained males and females implanted subcutaneously with cocoa butter, pineal powder or melatonin in various quantities. Group II contained males implanted hypothalamically with cocoa butter, pineal powder or melatonin in various quantities. Group III contained males and females subjected to bilateral orbital enucleation and subsequently electrochemically stimulated, or enucleated and sham stimulated.

Hamsters subjected to bilateral orbital enucleation showed a marked reduction of testicular weight. Electrochemical stimulation of the anterior, ventromedial, suprachiasmatic or arcuate hypothalamic nuclei, but not of the lateral hypothalamus or ventral thalamus of enucleated males, resulted in testicular and accessory sex organ weights above those of sham stimulated males. Enucleated females showed persistent vaginal diestrus. Following electrochemical stimulation in the arcuate nucleus, these females resumed normal estrous cycling earlier than nonstimulated females, and earlier than those stimulated in the lateral hypothalamus.

Implants of pineal powder or exogenous melatonin in the quantities employed did not significantly affect the

weights of the testes, male accessory sex organs studied, or the uteri of females or their estrous cycles. These effects are unlike those in rats. There was a clearly demonstrable antigonadal effect of pineal powder on the histological appearance of the testes of many males, and a palpable decrease in the size of a testis of certain males subcutaneously implanted with pineal powder.

The data render tenable the hypothesis of a hypothalamic site of action of a hamster pineal hormone. However, they do not eliminate the gonads as additional target organs. It may be that the hamster pineal antigonadal principle is a pineal polypeptide, rather than melatonin.

INTRODUCTION

Ambient lighting has been shown to affect the reproductive organs of rats, hamsters and mice. Continuous light increases the uterine weight and decreases the ovarian weight of rats (Bradshaw and Critchlow, 1966; Negro-Vilar, Dickerman and Meites, 1968; and Maric, Matsuyama and Lloyd, 1965). Exposure to continuous light also increases the incidence of vaginal estrus, as demonstrated by Browman (1937), Lawton and Schwartz (1965), and Prop and Ebels (1968) with mature female hamsters; and Chu, et al., 1964 with mature female mice. Exposure of female rats to continuous light increases the follicle stimulating hormone releasing factor content of the hypothalamus (Negro-Vilar, Dickerman and Meites, 1968). Lawton and Schwartz (1965 and 1967) and Maric, et al. (1965) have shown that exposure of female rats to continuous light for 30 to 60 days elevates the pituitary level of luteinizing hormone but that exposure to continuous light for longer periods depresses the pituitary level of luteinizing hormone and suppresses ovulation. Exposure of prepuberal female rats to continuous light induces hypertrophy of the puberal ovary and uterus (Wurtman, et al., 1961) induces precocious vaginal introitus (Fiske, 1941) and increases the incidence of postpuberal vaginal estrus (Chu, et al., 1964).

Subjecting rats and hamsters to short photoperiods (hence long periods of darkness daily), or to bilateral orbital enucleation produces certain effects on the reproductive organs. Some of these effects are opposite those produced by continuous light. Exposure of mature female rats to continuour darkness reduces the ovarian and uterine weights (Hoffman, 1967). Exposure of mature female hamsters to continuous darkness reduces the uterine but not the ovarian weight (Reiter, Hoffman and Hester, 1966; and Reiter, 1969). Exposure of adult male hamsters to continuous darkness reduces the weight of the testes and male accessory sex organs (Hoffman and Reiter, 1965; Reiter and Hester, 1966; Gaston and Menaker, 1967, and Reiter,1967, 1968 and 1969) and produces aspermia and atrophy of the seminiferous tubules (Reiter, 1969). Reiter, et al., 1968) have shown that exposure of prepuberal male rats to continuous darkness produces a decrease in the puberal testicular and accessory sex organ weights.

Pinealectomy has certain effects opposite those of continuous darkness and parallel to those of continuous light. Prepuberal pinealectomy of female rats elevates the puberal ovarian weight (Wurtman, et al., 1961). Pinealectomy of mature female rats followed by normal or continuous lighting increases the incidence of vaginal estrus (Gittes and Chu, 1965). Pinealectomy induces ovarian hypertrophy in adult female rats and this hypertrophy is blocked by pineal extracts (Wurtman, Altschule and Holmgren, 1959). Pinealectomy also prevents the depression of uterine weight observed in enucleated female hamsters (Reiter and Hester, 1966). In immature rats treated

with pregnant mare serum gonadotropin pinealectomy enhances ovulation (Dunaway and O'Steen, 1967). Kincl and Benagiano (1967) have shown that prepuberal pinealectomy induces precocious vaginal introitus.

Fraschini, Mess and Martini (1968a) and Reiter (1969) have shown that pinealectomy results in increasing the weight of the testes and accessory sex organs of adult male rats, and prevents the decrease in the weights of these organs in adult male hamsters exposed to continuous darkness. On the other hand, Motta, Fraschini and Martini (1967) could find no effect of pinealectomy on the testes of mature male rats. Reiter (1969) has shown that pinealectomy restores the testicular weight of male rats neonatally sterilized with testosterone. Pinealectomy of male rats elevates pituitary luteinizing hormone levels (Fraschini, et al., 1968a) and hastens the onset of copulatory behavior in rats exposed to continuous darkness during the prepuberal period (Baum, 1968).

Extracts of the pineal gland, or the pineal principle, melatonin (N-acetyl-5-methoxytryptamine), have certain effects on the reproductive organs. Some of these effects are opposite those of continuous light or pinealectomy and parallel to those of continuous darkness. Wurtman, et al. (1959) have shown that pineal extracts administered to prepuberal female rats reduced the puberal weight of the ovaries and (1961) inhibited ovarian hypertrophy in mature female rats exposed to continuous light during the prepuberal period. Pineal extracts (Wurtman, et al., 1959) or melatonin (Wurtman, Axelrod and Chu, 1963a) administered to prepuberal rats reduces the puberal ovarian

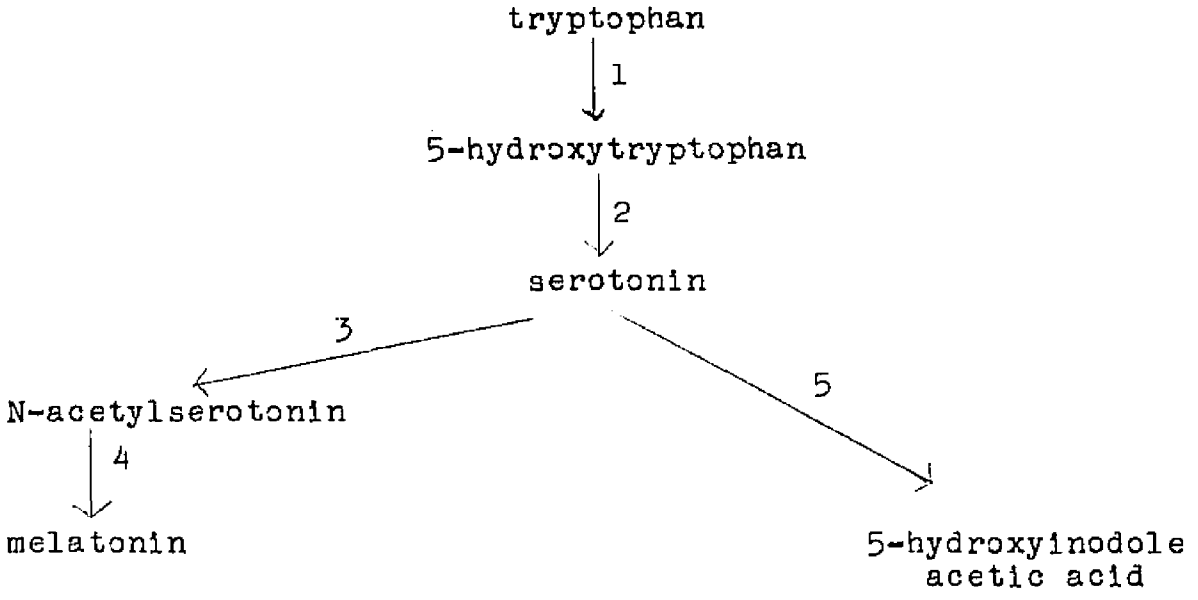
weight. Wurtman, et al. (1963a), Chu, Wurtman and Axelrod (1964) and Vaughan, O'Steen and Vaughan (1970) have shown that melatonin administered to neonatal or prepuberal female rats reduces the incidence of postpuberal vaginal estrus; and melatonin administered to female mice under continuous light postpones vaginal introitus (Chu, et al., 1964). When administered to prepuberal male rats melatonin reduces the puberal weights of the seminal vesicles and testes (Motta, et al., 1967) and, when administered to prepuberal male rats under continuous light, melatonin reduces the puberal weight of the testes and seminal vesicles (Debeljuk, 1969). Rust and Meyer (1969) have shown that melatonin reduces the testicular size of male weasels (Mustela erminea). Fraschini, et al. (1968a and b) have shown that melatonin implanted in the hypothalamus of castrated male rats reduces the plasma and pituitary levels of luteinizing hormone.

Pavel and Petruscu (1966) have shown that a highly purified pineal polypeptide blocks gonadotropin induced ovarian hypertrophy in immature female rats.

The pineal levels of certain pineal principles and the activities of certain pineal enzymes in the pathway for the conversion of tryptophan to melatonin or 5-hydroxyindole acetic acid have been correlated with ambient lighting. The pathway is presented in Figure 1. Light elevates and darkness depresses the pineal levels of 5-hydroxyindole acetic acid and serotonin (Quay, 1963 and 1964). Continuous light increases pineal 5-hydroxytryptophan decarboxylase activity and blinding prevents this response (Snyder, et al., 1965). Light

Figure 1

Pineal pathway for the conversion of tryptophan to melatonin or 5-hydroxyindole acetic acid



Steps	Enzymes	Reference
1	tryptophan hydroxylase	Lovenberg, et al. (1967)
2	5-hydroxytryptophan decarboxylase	Snyder, et al. (1965)
3	- - - - - - - - - - - - -	Weissbach, et al. (1960)
4	hydroxyindole-O-methyl transferase	Axelrod and Weissbach (1961)
5	monoamine oxidase, aldehyde dehydrogenase	Giarman, et al. (1959) Wurtman, et al. (1963) Wurtman, et al. (1968)

increases and darkness depresses the activity of pineal hydroxyindole-O-methyl transferase in rats (Wurtman, et al., 1963b and Axelrod, Wurtman and Snyder, 1965) and in hamsters (Anton-Tay and Wurtman, 1968).

Ariëns Kappers (1964) has shown that removal of the superior cervical sympathetic ganglion produces degeneration of the nervi conarii that innervate the rat's pineal gland. It is, therefore, probable that the pathway of photic information to the pineal in rats is via the superior cervical ganglion and the nervi conarii.

The preganglionic parts of this pathway have been examined. These parts consist of the retinas, optic nerves (Wurtman, et al.,1964a) and the inferior accessory optic tract (Axelrod, et al., 1966 and Moore, et al., 1967). Furthermore, transection or removal of the retinas, inferior accessory optic tract, or superior cervical ganglion prevents ovarian and uterine hypertrophy and the increased incidence of vaginal estrus observed in female rats exposed to continuous light (Wurtman, et al., 1964b and 1967). Removal of this ganglion blocks the atrophy of the reproductive organs observed in enucleated hamsters (Reiter, 1969).

The above suggests that the pineal gland mediates some of the effects of light on the reproductive organs by the synthesis or release of melatonin. In view of their demonstration that hypothalamic melatonin implants depressed the pituitary and plasma levels of luteinizing hormone in castrated male rats, Fraschini, et al. (1968a and b) have hypothesized that the hypothalamus is a site of action of melatonin. Since

the hypothalamic area implanted by Fraschini, et al. (1968a and b) also produces gonadotropin releasing factors (Flerko, 1963 and McCann, 1963), it is hypothesized that changes in the levels of melatonin mediate the effects of ambient lighting by suppressing hypothalamic synthesis or release of gonadotropin releasing factors when melatonin levels are high during darkness. Such a depression would induce lower levels of pituitary gonadotropins and thus cause atrophy of the reproductive organs.

To further examine this hypothesis, the effects of hypothalamic stimulation of enucleated hamsters and of subcutaneous or hypothalamic implants of pineal powder or melatonin in otherwise normal hamsters were examined in this study. If a pineal hormone acts at a site in accordance with the above hypothesis, one would expect that hypothalamic and, perhaps, subcutaneous implants of pineal extracts or of melatonin would elicit the gonadal atrophy observed in enucleated hamsters and that hypothalamic electrochemical stimulation of enucleated hamsters would reverse the effects of enucleation.

METHODS AND MATERIALS

Golden hamsters, Mesocricetus auratus, aged three to four months from the closed colony maintained by the Department of Zoology and Physiology at Louisiana State University in Baton Rouge were used in this investigation. The animals were kept in rooms maintained at approximatly 25° C and illuminated from 4 AM to 6 PM daily. Purina Laboratory Chow and tap water were available ad libitum at all times.

Nembutal Sodium (sodium pentobarbital, Abbott) was the anesthesia of choice. Before all surgical procedures and killing, each animal received intraperitoneally 0.20 ml of a 50 mg/ml solution (solvent: 40% propylene glycol, 10% ethyl alcohol, and 50% water). Whenever this injection was insufficient to induce anesthesia within ten minutes, additional injections of 0.05 ml each were given.

Each animal was placed in one of three groups. Group I consisted of males and females that received subcutaneous implants of tubes containing unadulterated cocoa butter, or mixtures of cocoa butter with pineal powder or melatonin described hereafter. Group II contained males that received hypothalamic implants of tubes containing unadulturated cocoa butter, or mixtures of cocoa butter with pineal powder or melatonin. A third group consisted of males and females that were enucleated and subsequently subjected to hypothalamic electrochemical stimulation or to sham stimulation.

Pineal powder for implantation was prepared by mixing 0.4 gm of bovine pineal powder (Nutritional Biochemical Corporation) with 4.0 gm of liquid cocoa butter. Melatonin for implantation was prepared in four dose levels. Levels A, B, and C were prepared by mixing, respectively, 0.00104, 0.00715, or 0.31000 gm of synthetic melatonin (Sigma Chemical Company) with 7.0 gm of liquid cocoa butter. Dose level D was prepared by mixing 0.21 gm of synthetic melatonin with 3.5 gm of liquid cocoa butter.

The substances to be implanted were drawn into glass capillary tubes 0.9 mm in diameter and 100.0 mm in length and stored at -6° C until the time of implantation.

Subcutaneous Implants

The ten subgroups of Group I (Table I) contain the 64 male and 42 female animals subcutaneously implanted. At the time of implantation tubes containing cocoa butter, pineal powder or melatonin were broken into segments 20 mm long and inserted under depilated skin in the lumbodorsal area of the trunk via an incision 2 cm long.

On the first day of the experiment animals in Subgroups I-1-a, I-1-b and I-1-c were implanted with one, two or three tubes of cocoa butter, respectively. One week later the animals were implanted on the contralateral side with the same number of tubes that they had initially received. Thereafter, until the day the animals were killed, each tube that had been in place for two weeks was replaced with a fresh one.

Animals of Subgroups I-2-a, I-2-b and I-2-c were

implanted initially with one, two or three tubes of pineal powder, respectively. One week later the animals were implanted on the contralateral side with the same number of tubes that they had initially received. Thereafter, until the day the animals were killed, each tube that had been in place for two weeks was replaced with a fresh one.

The animals of Subgroups I-3-a, I-3-b, I-3-c and I-3-d were implanted initially with one tube of melatonin in dose levels A, B, C or D, respectively. One week later the animals were implanted on the contralateral side with one tube of melatonin in a quantity equal to that which they had initially received. Thereafter, until the day the animals were killed, each tube that had been in place for two weeks was replaced with a fresh one.

The size of the testes of males was monitored weekly (i.e., on each day of implantation) by scrotal palpation. The estrous cycles of females were monitored by daily examination of the vaginal orifices for evidence of a postovulatory discharge. Males were killed when scrotal palpation indicated a decrease in the size of at least one testis when compared with normal males. In accordance with the experimental design females were to have been killed when there was a cessation of post ovulatory vaginal discharges, or when 12 weeks had elapsed since placement of the first subcutaneous implant, whichever came first. However, the estrous cycles remained regular and therefore all were killed 12 weeks after the initial implantation. At necropsy the testes, male accessory sex organs (coagulating glands, seminal vesicles and caudal

prostates, collectively) and the ovaries and uterine horns of females were removed. The testes and each uterine horn were blotted and weighed individually, and the male accessory sex organs were blotted and weighed collectively. One uterine horn from each female was frozen, lyopholized and reweighed. The unlyopholized uterine horns and both ovaries were placed in fixative. The male accessory sex organs were frozen, lyopholized and reweighed, collectively.

Hypothalamic Implants

Hypothalamic implantation in the 16 Group II males (Table II) was accomplished with the aid of a Model U Stereotaxic instrument manufactured by the Baltimore Instrument Company, Inc. and equipped with a headholder on a baseplate for small animals and a micrometer with divisions of two microns for accurate placement of the implant.

Scissors were used to remove the hair from the dorsum of the head over an area extending from the occiput to a point approximately 3.5 cm cephalad thereto. The surface of the skull was then exposed by a middorsal incision approximately 2.5 cm long extending cephalad from the occiput. The exposed skull in the incised area was then scraped with a scalpel to remove the galea aponeurotica. The hair was removed on each side of the head in the vicinity of the external opening of the external auditory meatus and the lateral wall of the meatus was incised sufficiently to accommodate the ear bars of the instrument.

Each animal was placed on the headholder of the baseplate so that the upper incisor bar was immediately posterior to the upper incisor teeth. The ear bars were inserted into the external auditory meatuses in such a position as to lie close to, or against the tympanic bullas. The upper incisor bar was positioned at a level 5 mm higher than the interaural line.

A tube containing the substance to be implanted was affixed to the electrode carrier of the stereotaxic instrument and positioned above the skull in accordance with the procedures described by Knigee and Joseph (1968). The electrode carrier was lowered until the tip of the tube was approximately 5 mm above the skull and the corresponding position on the skull was marked with a pencil. The electrode carrier was then raised and a dental drill was used to make a hole at the site marked. The electrode carrier was again lowered until the tip of the tube passed through the hole to assume a position in the area of the arcuate and ventromedial hypothalamic nuclei and the glass tube was broken at the surface of the skull. The animal was removed from the stereotaxic instrument and sulfadiazine powder (Lederle) was sprinkled around and into the field of operation and the incision was closed with wound clips or surgical sutures.

Fourteen days after implantation the brains, testes and male accessory sex organs were removed. The testes were blotted, weighed and placed in fixative. The male accessory sex organs were blotted, weighed, frozen, lyopholized and reweighed. The brains were placed in fixative for subsequent

microanatomical examination to confirm the location of the tip of the tube.

Enucleation and Electrochemical Stimulation

Forty-seven males and 13 females were bilaterally enucleated. Twelve weeks after enucleation all the males were hemicastrated. Thirty-five of the latter and eight of the 13 enucleated females were subjected to hypothalamic electrochemical stimulation. The remaining 12 enucleated hemicastrated males were subjected to sham stimulation, whereas the remaining 5 enucleated females were subjected to no additional surgical procedures.

To cause the eyeball to partially protrude from the orbit, the upper eyelid was raised and the lower lid was lowered, whereupon the tips of a pair of iris scissors were placed against the ventral aspect of the eyeball. By closing the scissors, the optic nerve, extrinsic eyeball muscles and other peribulbar tissues were severed. The eyeball was removed and discarded. The remaining orbital contents were cauterized.

In castrating the 47 males, the right testis was removed from approximately half the males and the left testis was removed from the remaining ones. Immediately thereafter the males were subjected to hypothalamic lesions that resulted in subsequent electrochemical stimulation.

Unipolar electrodes were prepared by immersing Number 2 stainless steel insect pins into Epoxylite 600-M insulator (The Epoxylite Corporation, South El Monte, California).

The pins were baked 30 minutes at 450° F. and after they had cooled a second coat of insulation was applied. The insulation at the tip of the pin was then removed with the aid of a dissecting microscope and a razor blade so that approximately 1 mm of the tip was exposed. Delivery of a current via a steel electrode results in the deposition of iron particles (Terasawa and Sawyer, 1969; Everett and Radford, 1961). Because of the production of a small lesion in the process, this procedure is often called "lesioning". However, subsequent electrochemical stimulation results from the residual iron particles and from the passage of current at the cathode. Bradshaw and Critchlow (1966) have shown that hypothalamic electrochemical stimulation can elevate pituitary luteining hormone levels. Hereafter, the terms lesion or stimulatory lesion will connote residual electrochemical stimulation.

The animals to be lesioned were placed on the stereotaxic instrument in the manner previously described. The electrode was affixed to the electrode carrier of the stereotaxic instrument and connected to the cathode of a direct current lesion producing device that provided fine adjustment control to 0.10 milliamperes and was manufactured by C. H. Stoelting Company. One ear bar of the stereotaxic instrument was connected to the anode of the lesion producing device. The tip of the electrode was positioned above the skull in accordance with the procedures described by Knigee and Joseph (1968). The electrode carrier was then lowered until the tip of the electrode was approximately 5 mm above the surface of the skull and the corresponding site on the

skull was marked with a pencil. A dental drill was used to make a hole at the marked site. The electrode carrier was again lowered until the tip of the electrode passed through the hole to the desired hypothalamic location. A current of 2.0 milliamperes was then applied for 6.0 seconds. For midsagittal lesions, the electrode was inserted once. Bilateral lesioning was accomplished by placing the electrode once through a left hole and once through a right hole. Sham lesioning was accomplished by insertion of the electrode without the passage of current. The electrode was withdrawn, the animal was removed from the stereotaxic instrument, and the wound was closed as described earlier.

Five weeks after lesioning the remaining testis and the male accessory sex organs were removed, blotted and weighed. The male accessory sex organs were frozen, lyopholized and reweighed. The testis was placed in fixative. The brain was removed and placed in fixative.

Eight enucleated females were subjected to stimulation at the end of the twelfth week after enucleation. No organs were removed prior to stimulation. The females were killed when they showed two or more regular estrous cycles after stimulation and the ovaries and brain were removed and placed in fixative. The uterine horns were removed, blotted and weighed individually. One of each female's uterine horns was frozen, lyopholized and reweighed. The other horn was placed in fixative.

The remaining five enucleated females were killed between the nineteenth and twenty-fourth weeks after enucleation.

The ovaries and uterine horns were collected and treated as described above. The brains were discarded.

Microantomical Procedures

The brains from lesioned animals were fixed for 48 hours or more in formol saline (Culling, 1963). To confirm the location of the lesion, the brains were frozen and sectioned at 50 microns and stained with Delafield's hematoxylin.

To confirm the position of implanted tubes, the brains from hypothalamically implanted animals were dissected under magnification.

The testes were fixed 48 hours or more, dehydrated, embedded in tissuemat, sectioned at 10 microns, and stained with Delafield's hematoxylin for subsequent histological examination.

Statistical Analyses

A completely randomized design analysis of variance was performed on all the data collected from male hamsters. When significant differences between treatments occurred, orthogonal comparisons were made among the treatments. The testicular weights of male hamsters subjected to electrochemical stimulation or sham stimulation were subjected to a covariance analysis to remove the effect of testicular weight collected at the time of electrochemical stimulation. The data collected from females were analyzed by the student's "t" test. A "p" value of less than .05 was accepted as the level of significance.

RESULTS

Subcutaneous Implants

The combined weights of the heavier and lighter testes, the combined weights of the accessory sex organs, the water content of the accessory sex organs, the weight of the lighter testes, the weight of the heavier testes, and the difference between the weight of the heavier and lighter testes were not significantly different among the 10 subgroups of subcutaneously implanted male hamsters (Tables III and IV).

The data derived from palpation of the testes (Table V.) provide an interesting contrast to those cited above. Of the eight males implanted initially with one tube of pineal powder, two showed a decrease in testicular size at the end of the fourth week (Table V, line 4) and four more showed a decrease by the end of the eighth week. The remaining two showed no palpable change in testes size by the end of the twelfth week, when they were killed. A palpable decrease in testicular size developed during the fifth week or later in 10 of the 38 males bearing more than one initial tube of pineal powder or bearing melatonin implants (Table V, lines 5 through 10). No palpable decrease in testicular size was observed in the 18 males implanted with cocoa butter alone.

No significant differences were observed when the wet uterine weights or the percentage of uterine water of females implanted subcutaneously with pineal powder or with melatonin were compared with the wet uterine weights or the percentage of uterine water of females implanted with cocoa butter alone (Table VI). Neither were there any abnormal alterations in the estrous cycles of the subcutaneously implanted females.

Hypothalamic Implants

Hypothalmic implants of pineal powder or melatonin in males in dose level A resulted in a greater difference between the heavier and lighter testes than did cocoa butter (Table VII, lines 2, 3 vs. 1). However, hypothalamic implants of melatonin in does level B resulted in a lesser difference between the heavier and lighter testes than did cocoa butter (Table VII, line 4 vs. 1).

The weights of the heavier testes from males implanted hypothalamically with melatonin in dose level A (Table VII, line 3) were significantly less than the weights of the heavier testes from males implanted hypothalamically with cocoa butter, pineal powder, or melatonin in dose level B (lines 1, 2, 4). The other hypothalamic implants resulted in no significant differences in the weights of the heavier testes.

The combined weights of the heavier and lighter testes, the combined weights of the accessory sex organs, and the water content of the accessory sex organs were not significantly different among the four subgroups of hypothalamically

implanted male hamsters (Table VIII). The weights of the lighter testes were not significantly different among the four subgroups of males hypothalamically implanted (Table VII).

Enucleated Electrochemically Stimulated Males

Twelve weeks after enucleation (i.e., on the day of electrochemical stimulation, where applicable the mean weight of the single testis collected from each of the 47 males was manifestly less than one half of the mean combined weights of the testes from 18 nonenucleated males receiving cocoa butter implants for the same period (.240 gm vs 1.382 gm/100 gm BW). At this time no palpable difference in size was observed between the two testes of any of the above animals.

The weight of the testes collected from males stimulated in the anterior hypothalamic area (AH), ventromedial nucleus (VM), suprachiasmatic nucleus (SC), or arcuate nucleus (Arc) was, at necropsy, greater than the weight of the testes collected from either sham stimulated males or from males stimulated in other areas (Table IX).

Both the wet and dry weights of the accessory sex organs of males stimulated in the AH, VM, SC or Arc nuclei were greater than those of the same organs collected from sham stimulated males (Table X). Furthermore, the wet and dry weights of the accessory sex organs of males stimulated in other areas were not significantly different from those

of the same organs collected from sham stimulated males (Table X).

Enucleated Electrochemically Stimulated Females

The enucleated females showed persistent vaginal diestrus by the end of the tenth week after encucleation. Twelve weeks after enucleation, five females were electrochemically stimulated in the lateral hypothalamus and three in the arcuate nucleus. Those stimulated in the arcuate nucleus resumed normal estrous cycling between the fifteenth and eighteenth weeks after enucleation. The five nonstimulated females and those stimulated in the lateral hypothalamus did not resume normal estrous cycling before the twentieth week after enucleation.

TABLES

Abbreviations Used in Tables

Abbreviation	Definition
AH	anterior hypothalamic area
Arc	hypothalamic arcuate nucleus
BW	body weight
EcS	electrochemical stimulation
F	females
gm	grams
M	males
N	number of animals
ns	not sugnificant
p	level of significance
SC	suprachiasmatic nucleus
SE	standard error
VM	ventromedial hypothalamic nucleus

Table I* Number and contents of initial tubes subcutaneously implanted in male and female hamsters.

Subgroups	N		Number of Tubes	Contents of Tubes
	M	F		
I-1-a	6	4	1	cocoa butter
I-1-b	7	4	2	" "
I-1-c	5	4	3	" "
I-2-a	8	4	1	mixture of pineal powder
I-2-b	9	6	2	" " " "
I-2-c	6	5	3	" " " "
I-3-a	6	0	1	melatonin does level A
I-3-b	6	4	1	" " " B
I-3-c	5	5	1	" " " C
I-3-d	6	6	1	" " " D

* - In this and the following tables see list of abbreviations.

Table II Number and contents of tubes implanted in the hypothalamus of male hamsters.

Subgroups	N	Number of Tubes	Contents of Tubes
II-1-a	4	1	cocoa butter
II-2-a	5	1	mixture of pineal powder
II-3-a	4	1	melatonin dose level A
II-3-b	3	1	" " " B

Table III Combined weight of heavier and lighter testes and of male accessory sex organs and water content of accessory sex organs of hamsters implanted subcutaneously with cocoa butter, pineal powder or melatonin.

Subgroup	N	Testes gm/100gm BW	(SE)*	Accessory sex organs gm/100gm BW	(SE)*	% water	(SE)*
I-1-a	6	2.7935	(.1881)	.5886	(.1168)	45.3	(10.9)
I-1-b	7	2.7197	(.1742)	.5758	(.1081)	43.5	(10.1)
I-1-c	5	2.7920	(.2061)	.4448	(.1279)	91.8	(11.9)
I-2-a	8	2.5242	(.1629)	.4061	(.1012)	60.1	(9.4)
I-2-b	9	2.3778	(.1536)	.5380	(.0954)	70.9	(8.9)
I-2-c	6	2.6038	(.1881)	.6555	(.1168)	44.8	(10.9)
I-3-a	6	2.5185	(.1881)	.4888	(.1168)	48.5	(10.9)
I-3-b	6	2.7068	(.1881)	.5235	(.1168)	60.5	(10.9)
I-3-c	5	2.7596	(.2061)	.7278	(.1279)	51.9	(11.9)
I-3-d	6	2.5278	(.1881)	.4562	(.1168)	50.6	(10.9)

* - Least-squares adjusted means.

Table IV Weight of the lighter and heavier testes and the weight difference between the heavier and lighter testes of male hamsters subcutaneously implanted with cocoa butter, pineal powder or melatonin.

Subgroup	N	Testes Weights* gm/100gm BW (SE)		Difference* gm/100gm BW (SE)	
		Lighter	Heavier		
I-1-a	6	1.3305 (.1477)	1.4630 (.1238)	.0127	(.0197)
I-1-b	7	1.2370 (.1367)	1.4827 (.1146)	.0240	(.0182)
I-1-c	5	1.3208 (.1618)	1.4712 (.1356)	.0146	(.0215)
I-2-a	8	0.8901 (.1278)	1.5966 (.1072)	.0702	(.0170)
I-2-b	9	1.0151 (.1206)	1.3627 (.1010)	.0342	(.0160)
I-2-c	6	1.0561 (.1477)	1.5477 (.1248)	.0485	(.0196)
I-3-a	6	1.0835 (.1477)	1.4350 (.1238)	.0347	(.0196)
I-3-b	6	1.0725 (.1477)	1.6343 (.1238)	.0557	(.0196)
I-3-c	5	1.0412 (.1618)	1.7184 (.1356)	.0670	(.0215)
I-3-d	6	1.0925 (.1477)	1.4355 (.1236)	.0338	(.0196)

* - Least-squares adjusted means.

Table V Number of males showing a palpable decrease in the size of at least one testis during the first eleven weeks after subcutaneous implantation of cocoa butter, pineal powder or melatonin.

Subgroup		N	Weeks 1-3	4	5	6	7	8	9	10	11	Total
1)	I-1-a	6										0
2)	I-1-b	7										0
3)	I-1-c	5										0
4)	I-2-a	8		2	1	1	1	1				6
5)	I-2-b	9			2				1			3
6)	I-2-c	6							1	1		2
7)	I-3-a	6					1					1
8)	I-3-b	6					1		1			2
9)	I-3-c	5							1			1
10)	I-3-d	6									1	1

Table VI Wet weights and water content of uteri from females implanted subcutaneously with cocoa butter, pineal powder or melatonin.

Subgroup	N	Uteri			
		Wet Weight gm/100gm BW (SE)*		% Water	Implanted Substance
1) I-1-a	4	.344	(.031)	82.9	cocoa butter
2) I-1-b	4	.277	(.028)	81.7	" "
3) I-1-c	4	.289	(.053)	80.1	" "
4) I-2-a	4	.350	(.036)	82.0	pineal powder
5) I-2-b	6	.287	(.039)	82.6	" "
6) I-2-c	5	.287	(.014	81.2	" "
7) I-3-b	4	.271	(.023)	82.2	melatonin
8) I-3-c	5	.302	(.032)	82.5	"
9) I-3-d	6	.291	(.032)	82.5	"

* - Simple mean

Table VII Weight of the lighter and heavier testes and the weight difference between the heavier and lighter testes of male hamsters hypothalamically implanted with cocoa butter, pineal powder or melatonin.

Subgroup	N	Testes Weights* gm/100gm BW (SE) Lighter	Heavier	Difference gm/100gm BW (SE)*
1) II-1-a	4	1.2412 (.1241)	1.7325 (.0700)	.0488 (.0098)
2) II-2-a	5	1.5114 (.1110)	1.5936 (.0626)	.0780 (.0088)**
3) II-3-a	4	1.3045 (.1241)	1.3668 (.0700)**	.0575 (.0098)**
4) II-3-b	3	1.5027 (.1433)	1.8113 (.0808)	.0303 (.0113)**

* - Least-squares adjusted means.

** - Significantly different from cocoa butter implants, line 1 ($p < .01$)

Table VIII Combined weight of the heavier and lighter testes and the weight and water content of the male accessory sex organs of hamsters implanted hypothalamically with cocoa butter, pineal powder or melatonin.

Subgroup	N	Testes gm/100gm BW	(SE)*	Accessory sex organs gm/100gm BW	(SE)*	% Water	(SE)*
1) II-1-a	4	2.9738	(.1761)	.3820	(.1572)	48.9	(10.6)
2) II-2-a	5	3.1050	(.1575)	.4262	(.1400)	49.3	(9.5)
3) II-3-a	4	2.6712	(.1761)	.4805	(.1572)	25.9	(10.6)
4) II-3-b	3	3.3143	(.2034)	.5293	(.1815)	65.7	(12.3)

* - Least-squares adjusted means.

Table IX Weight of testes five weeks after either EcS or sham stimulation.

Site of EcS	N	Testes Weight	(SE)*	p
Sham	12	0.4256	(.0568)	
AH, VM, SC or Arc	22	1.1847	(.0380)	<.01
Other nuclei**	13	0.3341	(.0348)	ns

* - Least-squares adjusted means.

** - Lateral hypothalamus and ventral thalamus.

Table X Wet and dry weights of the accessory sex organs of male hamsters five weeks after either EcS or sham stimulation.

Site of Ecs	N	Weights (SE)*				p**
		Wet		Dry		
Sham	12	.0948	(.0353)	.03133	(.0067)	
AH, VM SC or Arc	22	.3886	(.0240)	.0848	(.0045)	<.01
Other nuclei***	13	.1908	(.0339)	.0311	(.0064)	ns

* - Least-squares adjusted means.

** - p value applicable to both wet and dry weights.

*** - Lateral hypothalamus and ventral thalamus.

DISCUSSION

Bilateral orbital enucleation induced atrophy of the testes and accessory sex organs in the hamsters of this investigation and in those studied by Reiter in 1969. Furthermore, Reiter (ibid.) has shown that pinealectomy of enucleated hamsters prevented atrophy of these organs.

Following is a hypothetical sequence of hormonal events that may explain the atrophy of reproductive organs in enucleated male hamsters: (1) Enucleation induced an increase in the activity of the melatonin forming enzyme, hydroxyindole-O-methyl transferase, producing higher levels of the pineal hormone, melatonin. This is supported by the work of Anton-Tay and Wurtman (1968) who showed that darkness or enucleation stimulates the activity of this enzyme in hamster pineal glands. (2) High levels of melatonin act on the hypothalamus to suppress the synthesis and/or release of gonadotropin releasing factors produced by the AH, VM, SC and Arc nuclei. (3) The lower levels of gonadotropin releasing factors resulted in lower level of pituitary gonadotropins. (4) The lower levels of gonadotropins were inadequate to maintain the testes, which resulted in testicular atrophy. (5) The atrophied testes did not elaborate sufficient quantities of sex steriods to maintain the accessory sex organs, and atrophy ensued.

The data from the enucleated electrochemically stimulated males support the hypothetical sequence of hormonal events. The enucleated hamsters stimulated in hypothalamic areas that presumably secrete gonadotropin releasing factors had significantly greater testicular and wet and dry acessory sex organ weights than sham stimulated enucleated males or those stimulated in other nuclei. Hypothalamic electrochemical stimulation produces elevation of pituitary gonadotropin levels in rats, presumably by stimulating the output of gonadotropin releasing factors (Bradshaw and Critchlow, 1966). Therefore, it is likely that stimulation of these areas did, indeed, elevate gonadotropin levels, contravening the effect of the high levels of melatonin that are presumably present in enucleated male hamsters.

The proposed hypothetical sequence of hormonal events is also supported by the data collected from enucleated females. Enucleation induced a cessation of normal estrous cycling which is indicative of ovarian insufficiency. Electrochemical stimulation of enucleated females in the arcuate nucleus, but not in the lateral hypothalamus, resulted in a return to normal estrous cycles.

The facts that the decreased weight of the accessory male sex organs in enucleated males was restored by electrochemical stimulation in appropriate nuclei, and that ovarian cycles of enucleated females were similarly restored, are evidence that electrochemical stimulation contravenes the effect of a pineal antigonadal principle on the hypothalamus,

thereby inducing the elaboration of greater quantities of sex steriods.

Although the data from enucleated electrochemically stimulated hamsters support the hypothetical sequence of events, the data from administration of pineal powder and exogenous melatonin do not necessarily implicate melatonin as the active antigonadal pineal principal in hamsters. Pineal extracts or exogenous melatonin have been shown to cause a reduction in testicular weight in male rats and uterine weight in female rats. In the hamsters in this study, implants of pineal powder or melatonin in the quantities employed did not significantly affect the weights of the testes, male accessory sex organs studied, or the uteri of females or their estrous cycles. There was, however, a clearly demonstrable antigonadal effect of pineal powder on the histological appearance of the testes of many males; and, there was a palpable decrease in the size of at least one testis in 11 out of 23 males subcutaneously implanted with pineal powder. These observations suggest either that endogenous melatonin may have no effect on the reproductive organs of golden hamsters or that a quantity greater than that employed in this study must be administered to elicit effects comparable to those in other mammals. No reference to any effects of pineal extract or exogenous melatonin on the gonads of hamsters have been found in the literature. Since Pavel and Petrescu (1966) have found a polypeptide pineal principle which has antigonadal effects it may be that the hamster

pineal antigonadal principle is a pineal polypeptide, rather than melatonin.

The observations that hypothalamic implants of pineal powder or melatonin in certain dose levels significantly altered the difference between the weight of the heavier and lighter testes, and that hypothalamic implants of melatonin in dose level A, but not of pineal powder or of melatonin in does level B, lowered the weight of the heavier testes are inexplicable at this time, but cannot be ignored.

The data render tenable the hypothesis of a hypothalamic site of action of a hamster pineal hormone. However, they do not eliminate the gonads as additional target organs.

SUMMARY

1. The hamsters in this investagation were placed in three groups. Group I contained males and females implanted subcutaneously with cocoa butter, pineal powder or melatonin in various quantities. Group II contained males inplanted hypothalamically with cocoa butter, pineal powder or melatonin in various quantities. Group III contained males and females subjected to bilateral orbital enucleation and subsequently electrochemically stimulated, or enucleated and sham stimulated.

2. Hamsters subjected to bilateral orbital enucleation showed a marked reduction of testicular weight.

3. In enucleated males, electrochemical stimulation of the anterior, ventromedial, suprachiasmatic or arcuate hypothalamic nuclei, but not of the lateral hypothalamus or ventral thalamus, resulted in testicular and accessory sex organ weights above those of sham stimulated males.

4. Enucleated females showed persistent vaginal diestrus. Following electrochemical stimulation in the arcuate nucleus, these females resumed normal estrous cycling earlier than nonstimulated females, and earlier than those stimulated in the lateral hypothalamus.

5. Implants of pineal powder or exogenous melatonin in the quantities employed did not significantly affect the weights of the testes, male accessory sex organs studied, or the uteri of females or their estrous cycles. These effects are unlike those in rats.

6. There was a clearly demonstrable antigonadal effect of pineal powder on the histological appearance of the testes of many males, and a palpable decrease in the size of certain of the testes of males subcutaneously implanted with pineal powder.

7. The data render tenable the hypothesis of a hypothalamic site of action of a hamster pineal hormone. However, they do not eliminate the gonads as additional target organs.

8. It may be that the hamster pineal antigonadal principle is a pineal polypeptide, rather than melatonin.

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EXAMINATION AND THESIS REPORT

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Major Field: Zoology

Title of Thesis: Effect of Pineal Extracts and Hypothalamic Lesions on the Gonads of the Golden Hamster

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

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28 September 1970